ISOLATION AND CHARACTERIZATION OF PHYTOCHEMICALS FROM GARLIC SCALE

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DEPARTMENT OF BIOTECHNOLOGY AND MEDICAL ENGINEERING

C E R T I F I C A T E

This is to certify that the thesis entitled***"Isolation and Characterization of Phytochemicals from Garlic Scale"*** presented by Mr. Avinash Yamasani [Roll No 111BT0490] in fractional satisfaction of the prerequisites for the honour of the level of Bachelor of Technology in Biotechnology at National Institute of Technology, Rourkela is a bona fide work did by him under my direction. To the best of my insight the matter exemplified in the proposition has not been submitted to some other University/Institute for the grant of any degree or confirmation.

Date: 11th May 2015.

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Avinash Yamasani
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ABSTRACT

Garlic is a very useful bulbous plant whose bulbs have been researched and well known for its therapeutic uses. However garlic scales are less explored and their role unclear. In our study garlic scales were screened for various phytochemicals namely Coumarines, Quinines, Phlobotannnins, Tannins, Phenols, Cardiac Glycosides, Anthraquinones, Glycosides, Steroids, Terpenoids, etc. Numerous extraction methods were carried out such as soxhlet, cold extractions and hot extractions. Key highlights were cardiac glycosides and quinines observed in the garlic scales. When antibacterial assay was carried out using the garlic scale extract it was shown to inhibit the growth of *E.coli* significantly. Further cell viability was carried out using MG63 cells that showed that concentrations as low 100mg increased the proliferation ability of cells. Whereas higher concentrations proved to inhibit cell proliferation. Therefore garlic scales seem to contain phytochemicals that have significant biological functions that are to be studied further.

INTRODUCTION & LITERATURE REVIEW

Garlic

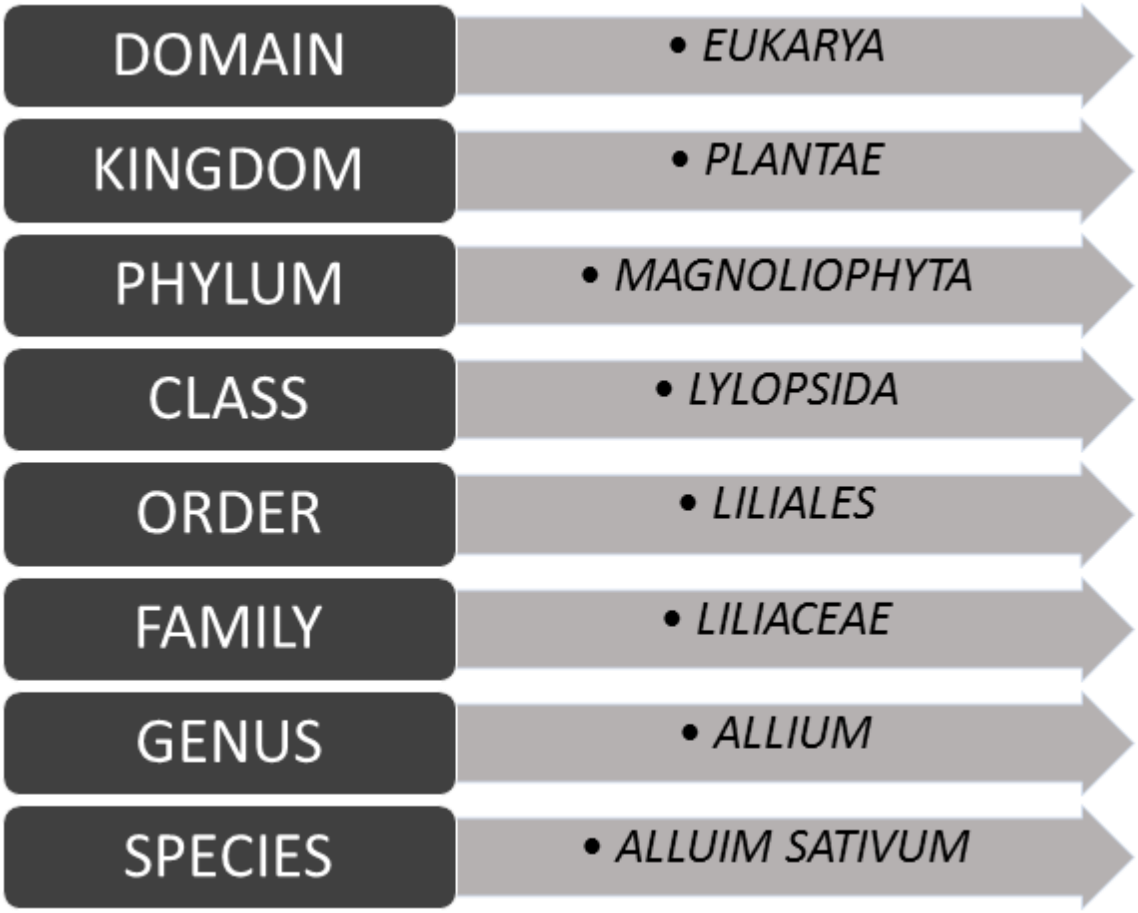
Allium sativum, commonly called garlic, falls under the category of the onion family termed Allium. *Allium sativum* is a bulbous plant which develops underground [1]. This has various therapeutic effects some known and others still remain unclear. Of recent Garlic has been utilized as part of heart and blood related disorders.

For instance, its use for alleviating hypertension, coronary heart assault, in cases of elevated cholesterol and symptoms like solidifying of veins. In addition Garlic's role in the treatment of several diseases like colon malignancy, prostate tumor, breast growth, and bladder disease cannot go unnoticed.

Several Sulphur containing compounds isolated from Garlic have been recognized in obliterating giloblastomas, a fatal mind tumor. Garlic is majorly incorporated in our day to day life to ameliorate common ailments such as normal colds and cerebral pains.

It has also been identified to fight against contagious infections and hostile to viral effects. Garlic has a unique component named Allicin which seems to account for most of its medicinal properties and numerous restorative properties furthermore this component has a huge market esteem even of recent times.

Taxonomy of Garlic



Phytochemicals

Phytochemicals are organically active, naturally occurring substances obtained from various plant species, which are known for their medicinal advantages to humans as compared to those credited to conventional macronutrients and micronutrients. They shield plants from sickness and ill effects while others are pigments that impart colour to the plant parts or have unique fragrances. Certain compounds that fall under the category of phytochemicals which are known to shield plant cells from ecological perils, for example, contamination, stress, drought, Ultra-Violet exposure and pathogenic attacks. With recent studies they have exhibited that it is obviously realized that they have potent roles in enhancing human wellbeing, which is usually identified only upon their deficiency in a person's daily diet [1].

Approximately 4,100 phytochemicals and more have been listed and are characterized by their biological function, physical nature and chemical properties additionally greater than 150 phytochemicals are analysed as potent drug target components.

Majorly phytochemicals are found in abundance as part of organic food products such as legumes, grains, herbs, spices, pulses, broccoli, cherries, wheat bread, tomatoes, beans cabbage onions, vegetables and garlic are basic sources. They gather in distinctive parts of the plants, for example, in blossoms and buds, natural seeds, stems, leaves, roots, tubers. Diverse phytochemicals, especially the coloured pigments, are frequently packed in external layers of different plant tissues. Levels fluctuate from plant to plant relying on the growth conditions, extent of processing, cooking and storage conditions [2].

Phytochemicals are majorly obtained as plant secondary metabolites produced in the stationary phase of growth when substrate conditions are limiting or cells are growing under stress, thus are not produced in positive correlation with growth rate. The role of phytochemicals as defensive compounds in plants has been proven to a great extent but confirmation is inadequate with regards to that they give the exact medical advantages as in humans and their roles in our biological microenvironment has to be studied.

These plant substances are known to have tremendous biological effects, for example, cell reinforcementaction, antimicrobial activity, esential part of antioxidant enzymes, abatement of platelet aggregation and regulation of hormone inactivation and anticancer effect [3]. There were greater than thousand known and numerous obscure phytochemicals which makes surely understood that plants create these chemicals to protect them, however recent findings show that numerous phytochemicals can likewise secure human against illnesses based on their key biological functions mentioned above.

Biological Activity of Phytochemicals

The phytochemicals produced in trees are in charge of avoiding malady and enhancing growth have been analysed broadly to overexpress them and to comprehend their activity. Studies include important steps such as isolation, characterisation and identification of components from various experimental plant species and corresponding cell line studies, experiments on animals models are done before the component can be put into clinical trials. Study discoveries propose phytochemicals diminish danger of cardiac (heart) illness by maintaining the oxidation of low density lipoprotein (LDL) cholesterol which aids in lessening the union or assimilation of cholesterol, normalizing pulse and coagulating activity of blood and enhancing blood vessel elasticity [4].

They seem to scavenge free radicals, hinder proteins that initiate cancer-causing agents, and actuate chemicals that detoxify cancer-causing agents. For instance, as indicated by information presented by Thomson, Genistein and Meagher phytochemicals tend to inhibit the arrangement of capillaries the step of angiogenesis that is required for tumour development and eventually metastasis [5]. The physiological properties of phytochemicals are surely known and high numerous exploration has concentrated on their conceivable part in counteracting and treating malignancy and coronary illness. Phytochemicals additionally advanced for the avoidance, treatment of diabetes, hypertension and macular degeneration. While phytochemicals are categorized according to their function, an individual compound can be identified to have numerous natural effects serving as an antioxidant and also as an antibacterial agent.

Classification of Phytochemicals

The accurate characterization of phytochemicals cannot be performed as such, due to the wide array of components that fall under this category. At present Phytochemicals are classified as primary or secondary components, based on their role in plant metabolism [6]. Primary constituents refer to the regular sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, photosynthetic pigments such as chlorophylls and so on. Secondary constituents are the remnant plant chemicals, for example, alkaloids, terpenes, curcumines, lignans, flavonoids, plant steroids, saponins, phenolics, and glucosides. Literature study show that phenolics are the most diverse Phyto constituents that majorly differ structurally.

Phenolics

Phenolic phytochemicals are the biggest class of phytochemicals and the most generally observed in the plant kingdom. The three most vital sources of dietary phenolics are flavonoids, phenolic acids, and polyphenols. Phenolic are made of a basic skeleton containing hydroxyl groups (-OH) attached to an aromatic hydrocarbon groups such as benzene ring. Phenol (C_6H_5OH) is viewed as the most basic component of this category of compounds. Generally, Phenolic substances are an expansive and complex group of mixture of constituents found in plants, it is rare that a particular plant species contains only one phenolic component [7].

They fall under the group of plant secondary metabolites, and they play an essential role as components in the plant defence mechanisms. Phenolic substances show certain unique properties that are considered valuable to people and its anti-cancer properties and tumour suppressing properties are due to their scavenging role against free radical. Thus they are hypothesized to be beneficial against free-radical mediated diseases [8].

Flavonoids are the largest group of plant phenols and relatively the most examined. Phenolic acids comprise of a differing collection of components that encompasses the broadly dispersed

hydroxybenzoic and hydroxycinnamic acids [9]. Phenolic polymers, usually known as tannins, are mixes of high sub-atomic weight that are divided into two classes: hydrolysable and dense tannins.

Phenolic Acids

The expression "phenolic acids", by and large, assigns phenols that have one carboxylic acid functional group. Characteristically happening phenolic acids contain two unmistakable carbon systems: the hydroxycinnamic and hydroxybenzoic structures [10]. Hydroxycinnamic acids are obtained as straightforward esters with glucose or hydroxy carboxylic acids Undoubtedly, tumor cells, including leukemia cells, commonly have larger amounts of reactive oxygen species (ROS) than typical cells with the goal that they are especially delicate to oxidative stress. Multiple papers and research work describe analyses on the bioavailability of phenolic acids, especially emphasizing both the direct uptake through food consumption and the indirect bioavailability obtained by gastric, hepatic and intestinal digestion [11].

Activity of Phenolic Acids

Phenolic substances are a well understood group of secondary metabolites with large pharmacological benefits. Phenolic acid substances and their corresponding functions have been the interest of an immense number of farming, organic, compound and medicinal field of studies. Phenolic substances in a variety of plants are polymerized into bigger particles, for example, the pro-anthocyanidins and lignins. In addition, phenolic acids may emerge in food crops as glycosides or esters with other identical components, for example, sterols, alcohols, glucosides and hydroxyl fatty acids [12]. Numerous natural functions of phenolic acids were analysed and reported correspondingly. For instance, their role in increased bile discharge, blood cholesterol reduction capacity as well as certain lipid levels and importantly antimicrobial action against a few strains of microbes.

Flavonoids

Flavonoids are polyphenolic components that are common in nature. More than 4,000 flavonoids have been studied, a considerable lot of which are available in vegetables, products like tea, coffee and natural fruit drinks. The flavonoids seem to have assumed a real part in successful therapeutic medications of ancient times, and their utilization has endured up to now. Flavonoids are pervasive among vascular plants and happen as aglycones, glucosides and methylated subordinates [13]. More than 4000 flavonoids have been depicted so far inside the parts of plants ordinarily devoured by people and roughly 650 flavones and 1030 flavanols are known. Most flavonoids happen characteristically connected with sugar in conjugated structure and, inside any one class, may be described as monoglycosidic, diglycosidic, and so forth [14].

Activity of Flavonoids

Flavonoids have increased late consideration due to their expansive organic and pharmacological exercises in these request Flavonoids have been accounted for to apply numerous natural property including antimicrobial, cytotoxicity, mitigating and also antitumor exercises yet the best-portrayed property of each gathering of flavonoids is their ability to go about as effective cancer prevention agents which can shield the human body from free radicals and receptive oxygen species [15]. The limit of flavonoids to go about as cell reinforcements relies on their atomic structure. The position of hydroxyl gatherings and different highlights in the concoction structure of flavonoids are essential for their cancer prevention agent and free radical searching exercises. Then again flavonoids, for example, luteolin and cathechins, are preferred cancer prevention agents over the supplements cell reinforcements, for example, vitamin C, vitamin E and β-carotene. Flavonoids have been expressed to have numerous valuable properties, containing calming movement, chemical restraint, antimicrobial action, and oestrogenic movement, hostile to hypersensitive movement, cancer prevention agent

action, vascular movement and cytotoxic antitumor action. Flavonoids constitute an extensive variety of substances that assume critical part in securing organic frameworks against the hurtful impacts of oxidative methods on macromolecules, for example, starches, proteins, lipids and DNA [16].

Tannins

From a chemical perspective it is hard to characterize tannins since the term includes some exceptionally differing oligomers and polymers. It may be said that the tannins are a heterogeneous gathering of high sub-atomic weight polyphenolic mixes with the ability to shape reversible and irreversible buildings with proteins (essentially), polysaccharides, alkaloids, nucleic acids and minerals, and so on [17]. On the premise of their structures qualities it is in this manner conceivable to partition the tannins into four noteworthy gatherings: Gallotannins, ellagitannins, complex tannins, and consolidated tannins.

- Gallotannins are every one of those tannins in which galloyl units or their meta- depsidic subsidiaries are certain to various polyol-, catechin-, or triterpenoid units.
- Ellagitannins are those tannins in which no less than two galloyl units are C–C coupled to one another, and do not contain a glycosidically connected catechin unit.
- Complex tannins will be tannins in which a catechin unit is bound glycosidically to a gallotannin or an ellagitannin unit.

Activity of Tannins

In prescription, particularly in Asian (Japanese and Chinese) characteristic mending, the tannin-containing plant concentrates are utilized as astringents, as diuretics, against stomach and duodenal tumors, and as mitigating, germicide, cancer prevention agent and haemostatic pharmaceuticals and against diarrhoea. Tannins are utilized as a part of the dyestuff business as caustics for cationic colors (tannin colors), furthermore in the creation of inks (iron gallate

ink) [18]. In the sustenance business tannins are utilized to clear up wine, brew, and organic product juices. Other modern employments of tannins incorporate material colors, as cell reinforcements in the natural product squeeze, lager, and wine commercial enterprises, and as coagulants in elastic Production [19]. As of late the tannins have pulled in experimental hobby, particularly because of the expanded rate of dangerous diseases, for example, AIDS and different malignancies. The quest for new lead mixes for the improvement of novel pharmaceuticals has ended up progressively essential, particularly as the organic activity of tannin-containing plant concentrates has been all around reported [20].

Terpenoids

The terpenoids are a class of characteristic items which have been gotten from five-carbon isoprene units. A significant number of the terpenoids are economically intriguing due to their utilization as flavors and aromas in nourishments and makeup samples menthol and sclareol or in light of the fact that they are imperative for the nature of agrarian items, for example, the kind of leafy foods scent of blooms like linalool [21]. The greater part of the terpenoids have multi cyclic structures that contrast from each other by their utilitarian gatherings and essential carbon skeletons [22]. These sorts of regular lipids can be found in every class of living things, and in this way considered as the biggest gathering of characteristic items. Terpenes are boundless in nature, for the most part in plants as constituents of crucial oils [23]. Their building piece is the hydrocarbon isoprene, $CH_2=C(CH_3)-CH=CH_2$. Terpene hydrocarbons subsequently have atomic formula (C_5H_8) n and they are characterized by number of isoprene units [24].

Activity of terpenoids

Among plant auxiliary metabolites terpenoids are a fundamentally most various gathering; they work as phytoalexins in plant direct barrier, or as signs in backhanded guard reactions which includes herbivores and their regular adversaries [25]. Numerous plants produce unpredictable terpenes keeping in mind the end goal to pull in particular bugs for fertilization or generally to oust certain creatures utilizing these plants as sustenance. Less unpredictable however firmly biting tasting or dangerous terpenes additionally shield a few plants from being eaten by creatures (anti fedants) [26]. Last, yet not minimum, terpenes assume a critical part as sign mixes and development controllers (phytohormones) of plants, as demonstrated by preparatory examinations. Furthermore, terpenoids can have restorative properties, for example, anticarcinogenic (e.g. perilla liquor), antimalarial (e.g. artemisinin), hostile to ulcer, hepaticidal, antimicrobial or diuretic (e.g. glycyrrhizin) movement and the sesquiterpenoid antimalarial medication artimisinin and the diterpenoid anticancer medication taxol [27].

Cardiac Glycosides

Cardiac glycosides represnt to a group of intensifies that are gotten from the foxglove plant. Cardiac glycosides can be termed as the organic compounds which contains sugars which acts on the cardiac muscles . They are highly toxic substances [28]. They have the ability to effect the function of heart. From old times, people have utilized cardiac-glycoside-containing plants and their rough concentrates as bolt coatings, destructive or self-destructive guides, rodent harms, heart tonics. In present day times, decontaminated concentrates or manufactured analogs of a couple have been adjusted for the treatment of congestive heart failure and cardiac arrhythmia [29].

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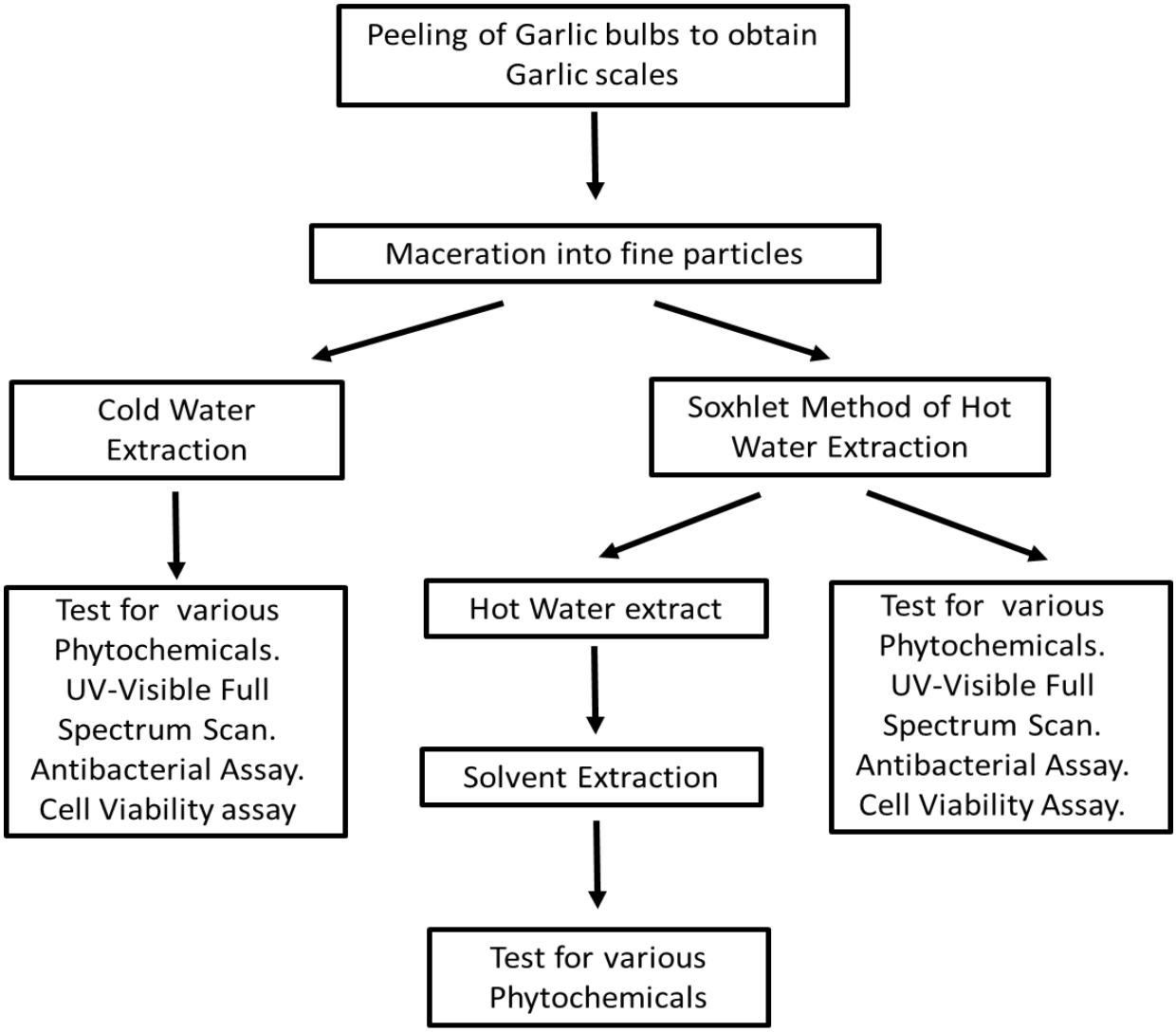
OBJECTIVE:

1. To extract the phytochemicals using
    - Simple maceration technique at 37˚C.
    - Cold assisted maceration technique.
    - Soxhlet extraction technique.
2. To study the Anti-bacterial activity of hot & cold extracts.
3. To perform MTT assay to study the cell proliferative index

WORK PLAN



MATERIALS AND METHODS

Materials

All the chemicals utilized for the examination were sterile and of standard configuration. For Garlic skin treatment, chemicals utilized were procured from HI media. For protein extraction Chemicals were obtained from Sigma-Aldrich.

Methodology

Preparation of Garlic samples

Fresh garlics were acquired from the market. The garlic scales were peeled off deliberately, staying away from the breakage of skins. The garlics were cleaned with refined water thrice before utilization. From that point, Garlic scales were treated with 0.1 N NaOH, ethanol, acetone and water, by drenching them every dissolvable and hatching for 24 hours at 37^{o}C as per the prerequisites of different tests. For organic studies, it was treated with Phosphate Buffer Saline, for 24 hours, for standardization. Garlic skins were powdered utilizing processor for MTT assay.

Phytochemical Screening

a) Test for Cardiac glycosides (Keller Kelliani's test)

- 5ml of every concentrate was treated with 2ml of H_2SO_4 in a test tube and a drop of ferric chloride arrangement was added to it. This was deliberately underlayed with 1ml concentrated sulphuric corrosive. A Brown ring may form that indicates the presence of deoxysugar normal for cardenolides.

b) Test for Flavonoids (Alkaline reagent test)

- 2ml of concentrates was treated with couple of drops of 20% sodium hydroxide arrangement. Arrangement of exceptional yellow shading, which gets to be coloueless on adding hydrochloric acid, demonstrates the vicinity of flavonoids.

c) Test for Phenols (Ferric chloride test)

- A small amount of the concentrates was treated with watery 5% ferric chloride and watched for development of dark blue or dark colour.

d) Test for Phlobatannins (Precipitate test)

- 2ml of concentrate was overflowed with 1ml of 1% watery hydrochloric acid and the formation of red coloured precipitate indicates the presence of Phlobotannins.

e) Test for Saponins (Foam test)

- To 2ml of sample was included 6ml of water in a test tube. The mixture was shaken energetically and watched for the development of persevering froth that affirms the vicinity of saponins.

f) Test for Sterols (Liebermann-Burchard test)

- 1ml of sample was treated with drops of chloroform, acidic anhydride and conc. H_2SO_4 and watched for the development of dark pink or red shading.

g) Test for Tannins (Braymer's test)

- 2mls of concentrate was treated with 10% alcoholic ferric chloride arrangement and watched for development of blue or greenish shading arrangement.

h) Test for Terpenoids (Salkowki's test)

- 1ml of chloroform was added to 2ml of every concentrate took after by a couple of drops of concentrated sulphuric corrosive. A ruddy cocoa accelerate created instantly demonstrated the vicinity of terpenoids.

i) Test for Quinones

- A little measure of concentrate was treated with concentrated HCL and watched for the arrangement of yellow precipitate (or colouration).

Antimicrobial Assay

- 7gm of Nutrient Agar was weighed and added to 250ml of distilled water in a conical flask and autoclaved prior to use.
- 20ml of the autoclaved media is dispensed into 2 sterile petri dishes in a laminar air flow chamber and left to cool down and solidify.
- The plates were incubated overnight at 37^{o}C and check the bacterial states if no bacterial culture develops the plates are sterile.
- Three equidistant wells are cut out using a micro pipette tip and labelled appropriately. 2 pairs were prepared to serve as duplicates.
- One well is taken as positive control and loaded with 500µl of 1mg.ml^{-1} Metronidazole a known antibiotic drug to work effectively against anaerobic bacteria.
- Other two wells were loaded with 500µl of 1mg.ml^{-1} of cold extract and hot extract respectively.
- After loading the wells 1ml of E.coli culture was added and spread using a sterile L shaped rod.
- The plates were sealed with parafilm and left in 30^{o}C overnight.
- The following day the plates were examined for zone of inhibition.

Soxhelet Method

Preparation of Sample

- Garlic is peeled, washed with distilled water and spread in a long tray and placed in the incubator for full drying.
- The dried peel of garlic is then made to fine powder using a grinder.
- Then it was enclosed in filter paper and put in the soxhelet apparatus.

- Water is poured into the solvent flask.
- The extraction is done at a temperature of 100 to 110^{o}C and then repeated for 10-15 cycles.
- Make the solvent in the flask to evaporate and get condensed in the extractor.
- The solvent in the flask gets concentrated solution containing the sample.
- The solution is taken out cooled and poured into petri plates.
- The petri plates were put in the incubator and dried.
- The dried plates were taken out and scraped it out.

Solvent Extraction

- 10 mg of the powdered sample which was got from scraping out the plate is weighed and put in 20ml of water.
- The solution is poured into the separating funnel and diethyl ether was added to it.
- The funnel was left without disturbing for 4 hours at room temperature.
- Then there will be the formation of two layers of immiscible liquids.
- Organic substances are concentrated in the organic solvents from water.
- The concentrated organic solvent i.e., diethyl ether is drained out from the funnel and collected in the falcon tube.
- The organic solvent is carried out for phytochemical screening.

UV Spectrophotometer Spectrum Scan

- 5mg and 10 mg of the sample is weighed and dissolved in water to make two different solutions of 5mg/ml and 10 mg/ml.
- Then the two solutions were half diluted and put in the cuvettes of the spectrophotometer.
- The wavelength was set to 200-790 nm and the scanning was carried out.

Seeding of MG63 cells:

- 5 $X10^4$ cells were seeded into each well of a 24 well plate. 300 µl of DMEM media was added to each well. The cells were cultured in an incubator at 37˚C and 5% CO_2 for one day. 100µg/ml and 10µg/ml were added to the wells. Wells without any sample were taken as control.

Cell viability assay

- After 24 hours of cell seeding, 20 µl of the MTT reagent was added to each well.
- Cells were incubated in an incubator at 37˚C and 5% CO_2 for 3 hours.
- The media was removed then and 200 µl DMSO was added to each well to dissolve the purple colour formazan crystals formed after the incubation.
- The optical density was measured at 595 nm.

RESULTS

- The following results are for the phytochemical screening of solution obtained after maceration technique.

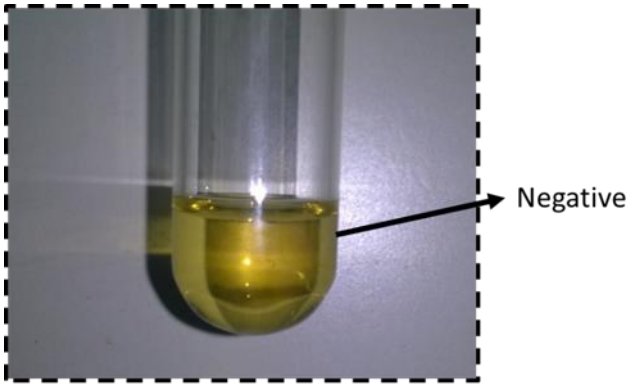


Fig.1 Coumarines

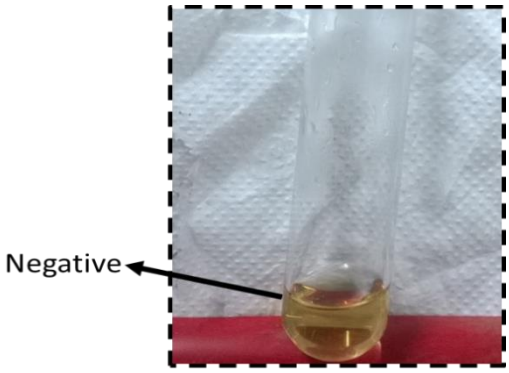


Fig.2 Quinines

Phytochemical name	Identification	Observation	Result
Quinines	Red colour	Red colour	Negative
Coumarines	Yellow colour	No colour	Negative

Table:1 Identification Of Quinines and coumarines of cold garlic extracts

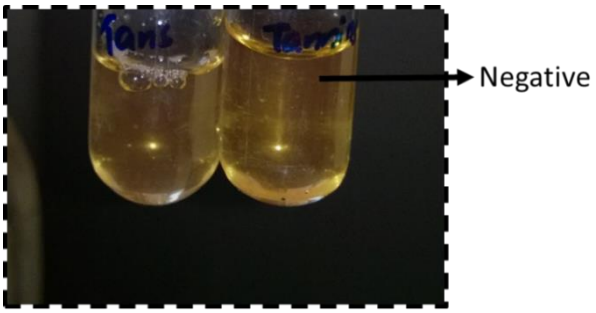


Fig.3 Tannins

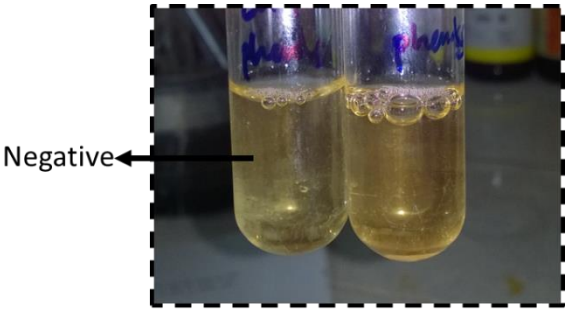


Fig.4 Phenols

Phytochemical name	Identification	Observation	Result
Tannins	Greenish Blue colour	Orange colour	Negative
Phenols	Blue or Green colour	Pale yellow colour	Negative

Table:2 Identification Of Tannins and Phenols of cold garlic extracts

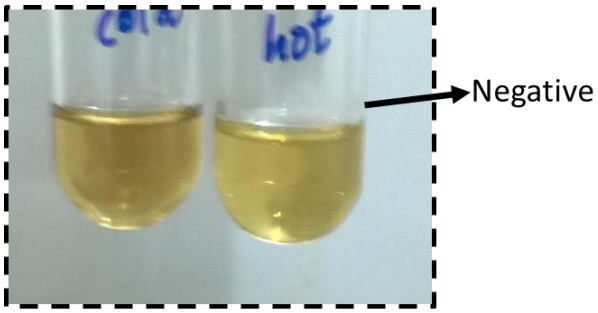


Fig.5 Phlobotannins

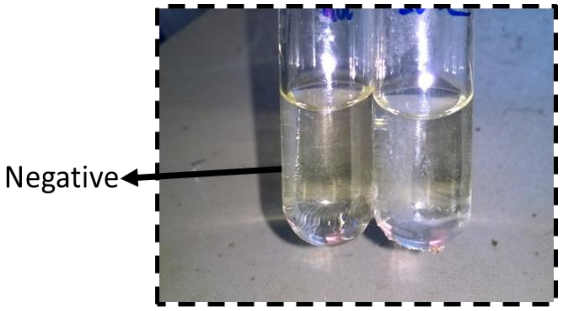


Fig.6 Cardiac glycosides

Phytochemical name	Identification	Observation	Result
Phlobotannins	Pink colour	No colour	Negative
Cardiac Glycosides	**Brown ring**	**Brown ring**	**Positive**

Table:3 Identification Of Phlobotannins and Cardiac Glycosides of cold garlic extracts

- The results obtained for the various phytochemical screening after the sample concentrated with Soxhlet extraction method tests are given below.

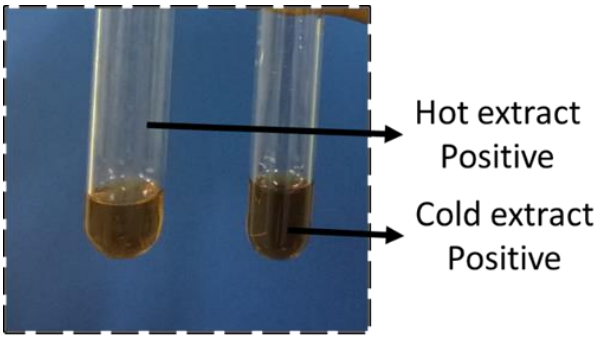


Fig.7 Qunines

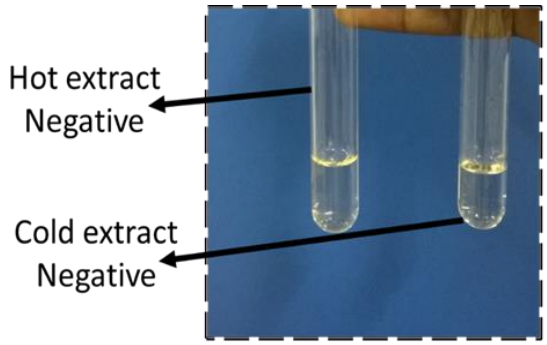


Fig.8 Anthroquinones

Phytochemical name	Identification	Observation	Result
Quinines	**Red colour**	**Red colour**	**Positive**
Anthroquninoes	Pink colour	Pale yellow colour	Negative

Table:4 Identification Of Quinines and Anthroquniones of hot garlic extracts

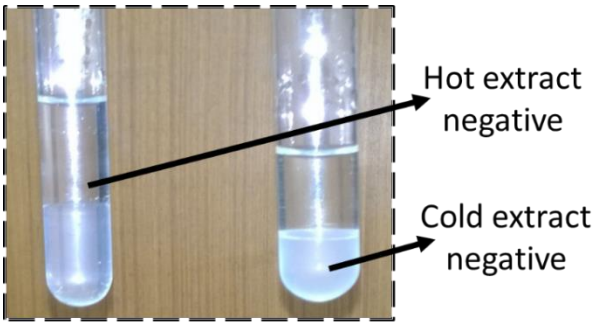


Fig.9 Glycosides

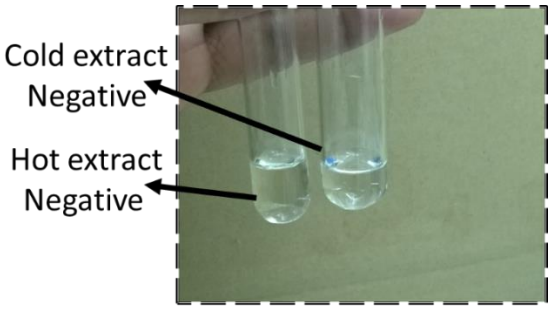


Fig.10 Coumarins

Phytochemical name	Identification	Observation	Result
Glycosides	Pink colour	No colour	Negative
Coumarines	Yellow colour	No colour	Negative

Table:5 Identification Of Glycosides and Coumarines of hot garlic extracts

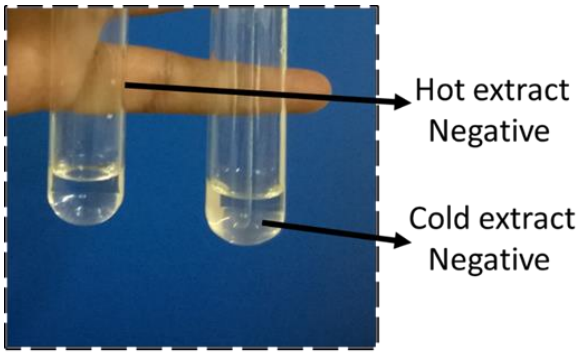


Fig.11 Steroids

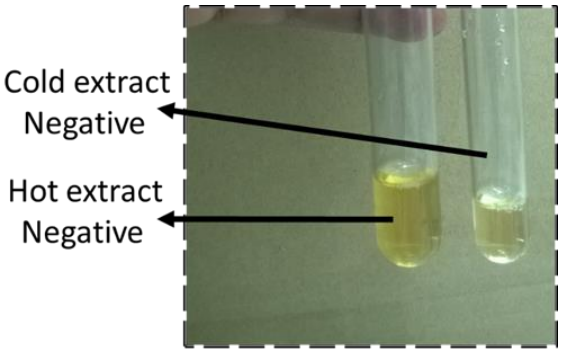


Fig.12 Phenols

Phytochemical name	Identification	Observation	Result
Steroids	Brown Ring	No colour	Negative
Phenols	Blue or Green colour	Pale yellow colour	Negative

Table:6 Identification Of Steroids and Phenols of hot garlic extracts

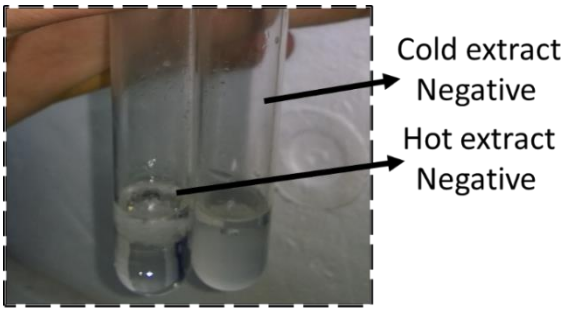


Fig.13 Terpenoids

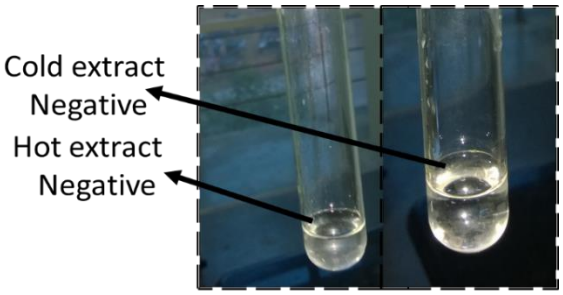


Fig.14 Phlobotannins

Phytochemical name	Identification	Observation	Result
Terpenoids	Red brown colour interface	No colour	Negative
Phlobotannins	Pink colour	No colour	Negative

Table:7 Identification Of Terapenoids and Phlobotannins of hot garlic extracts

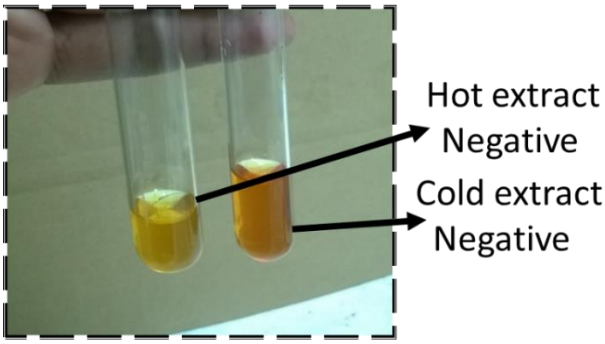


Fig.15 Tannins

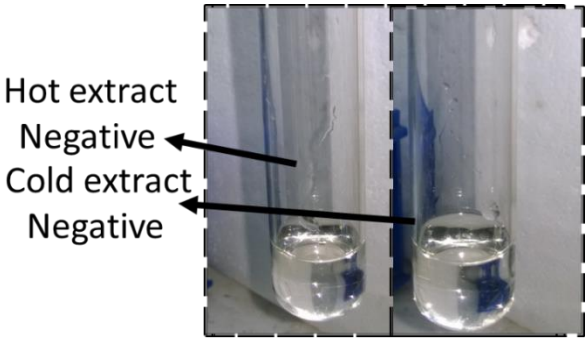


Fig.16 Flavanoids

Phytochemical name	Identification	Observation	Result
Tannins	Greenish Blue colour	Orange colour	Negative
Flavanoids	Yellow colour	No colour	Negative

Table:8 Identification Of Tannins and Flavanoids of hot garlic extracts

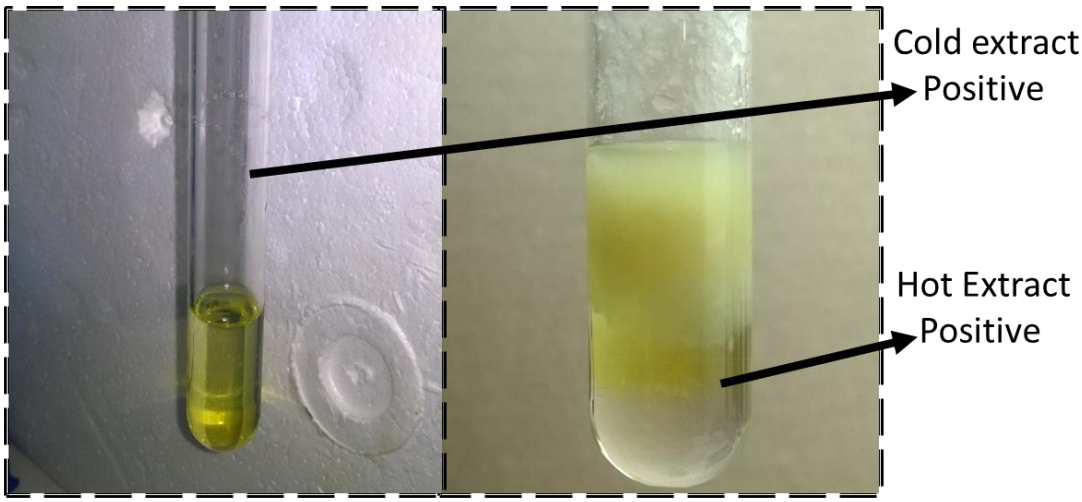


Fig.17 Cardiac Glycosides

Phytochemical name	Identification	Observation	Result
Cardiac Glycosides	Brown ring	Brown ring	Positive

Table:9 Identification Of Cardiac Glycosides of hot garlic extracts

- Solvent extraction was further carried out on the concentrated powder obtained from Soxhlet method of extraction and various phytochemical screening carried out but only Cardiac Glycosides were present recurrently.

Fig.18 Solvent Extraction Apparatus

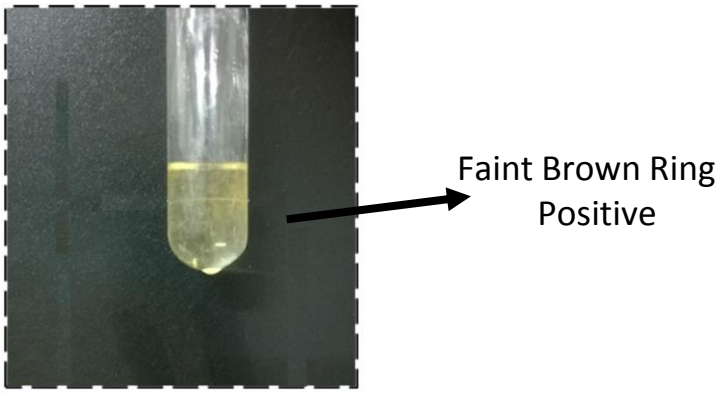


Fig.19 Cardiac Glycosides

- Consolidated representation of the various phytochemicals tested for and their observed results.

Phytochemical name	Identification	Observation	Result
Quinines	**Red colour**	**Red colour**	**Positive**
Anthroquninoes	Pink colour	Pale yellow colour	Negative
Glycosides	Pink colour	No colour	negative
Cardiac Glycosides	**Brown ring**	**Brown ring**	**Positive**
Coumarines	Yellow colour	No colour	Negative
Steroids	Brown ring	No colour	Negative
Phenols	Blue or Green colour	Pale yellow colour	Negative
Flavanoids	Yellow colour	No colour	Negative
Terpenoids	Red brown colour interface	No colour	Negative
Phlobotannins	Pink colour	No colour	Negative
Tannins	Greenish Blue colour	Orange colour	Negative

Table:10 Consolidate analysis of phytochemical screening

- For both the dry powder extracted from cold extract and hot extract were tested for antibacterial activity and the results obtained were.

Fig.20 Zone of Inhibition

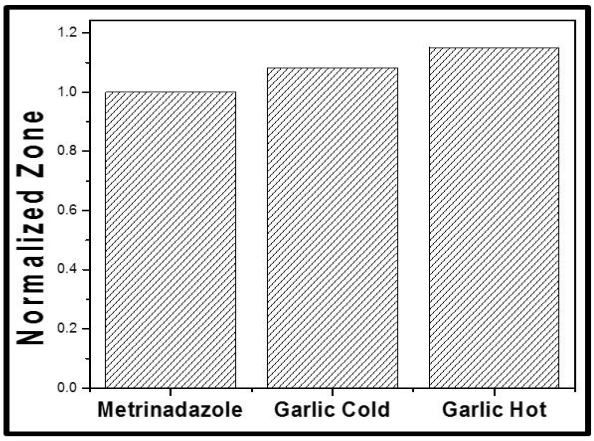


Fig.21 Graphical representation of zone of inhibition

S.No	Zone Area (cm^2)	Radius (cm)	Normalized Zone
1	3.812	1.101822	1
2	4.128	1.146582	1.082896
3	4.389	1.182274	1.151364

Table:11 Area and radius of zone of inhibition of garlic scale extracts

UV Spectrophotometer analysis of garlic scale extracts:

S.No	Sample	Peaks	Absorbance
1	**Cold extract**		
	10mg.ml^{-1}	422.0 333.2	2.586 3.048
	5mg.ml^{-1}	311.6	3.022
2	**Hot Extract**		
	10mg.ml^{-1}	333.2	3.048
	5mg.ml^{-1}	291.2	3.006

Table:12 UV spectroscopic analysis of garlic scale extracts

Cell Viability assay:

- Further Cell Proliferation Assay was carried out using 1mg.ml^{-1} and 100µg.ml^{-1} concentrations of both cold and hot extracts.

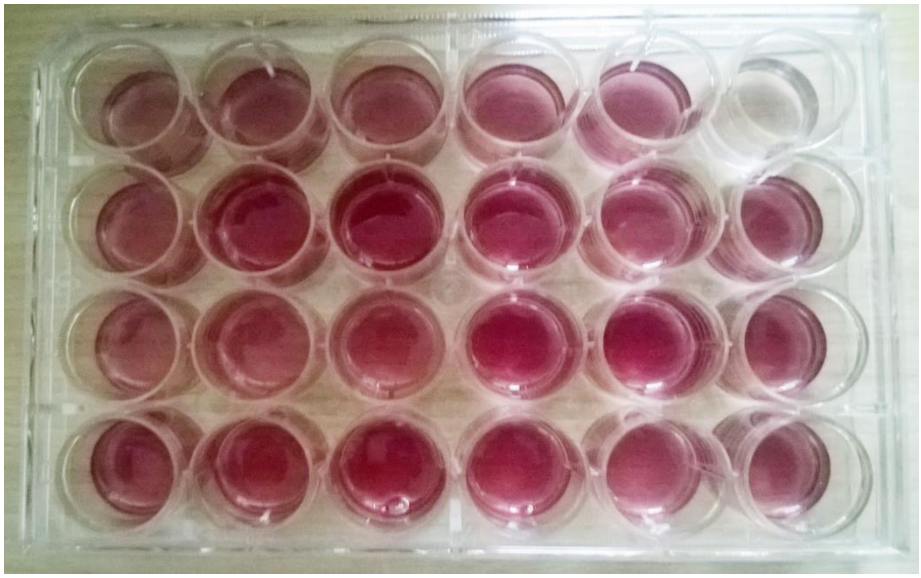


Fig.22 24 well plate showing MTT Assay carried out for different concentration of sample

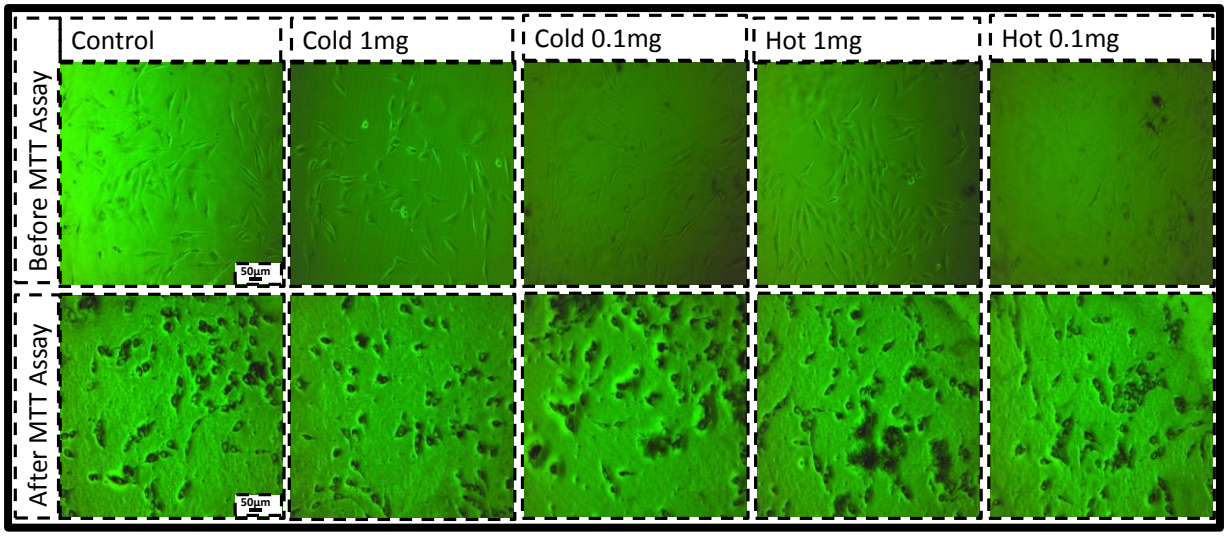


Fig.23: Cell viability analysis of different samples using MTT assay

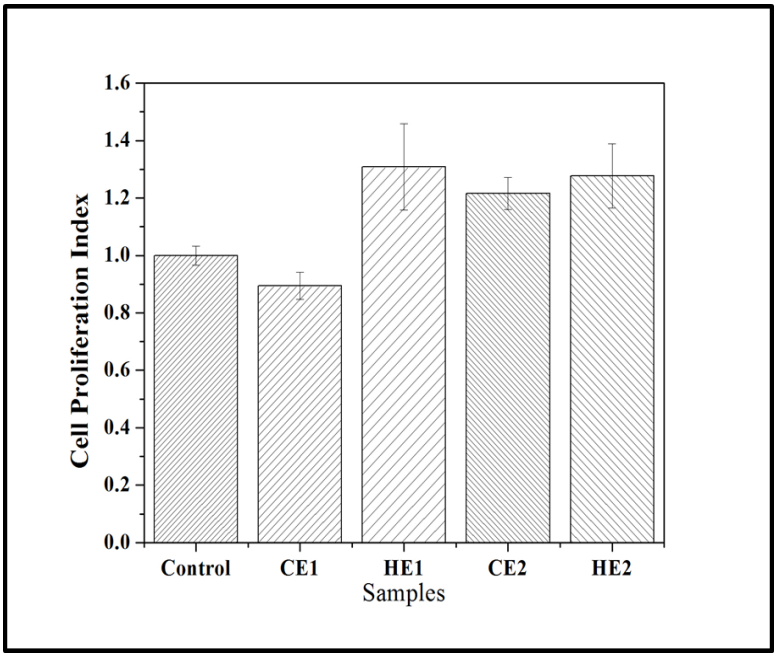


Fig.24 Cell proliferation index of garlic scale extracts. CE1 and CE2 – 1mg/ml and 100mg/ml of cold extracts. HE1 and HE2 – 1mg/ml and 100mg/ml of hot extracts. All experiments were done in triplicates.

CONCLUSION

Garlic is an extremely helpful bulbous plant whose globules have been examined and surely understood for its restorative employments. However garlic scales are less investigated and their part misty. In our study garlic scales were screened for different phytochemicals to be specific Coumarines, Quinines, Phlobotannnins, Tannins, Phenols, Cardiac Glycosides, Anthraquinones, Glycosides, Steroids, Terpenoids, and so forth. Various extraction strategies were done, for example, soxhlet, cold extractions and hot extractions. Key highlights were heart glycosides and quinines were seen in the garlic scales. At the point when antibacterial test was done utilizing the garlic scale separate it was demonstrated to repress the development of E.coli essentially. Further cell proliferation was completed utilizing MG63 cells that demonstrated that fixations as low 100mg expanded the multiplication capacity of cells. Though higher focuses demonstrated to restrain cell multiplication. Hence garlic scales appear to contain phytochemicals that have huge organic capacities that are to be contemplated further.

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